EFFECTS OF THERMAL PRE-TREATMENT ON ANAEROBIC DIGESTION OF NANNOCHLOROPSIS SALINA BIOMASS FOR BIOGAS PRODUCTION

by

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ABSTRACT

Effects of thermal pre-treatment on anaerobic digestion of *Nannochloropsis salina* biomass for biogas production

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Nannochloropsis salina is looked at as an exemplary species for anaerobic digestion because of its high biomass productivity and its high lipid content. Although considered a model species, cell wall degradation of N. salina is a limiting factor for efficient anaerobic digestion, but can be overcome with pre-treatment. The objective of this experiment was to test the effects of thermal pre-treatment by autoclaving at 121°C for *Nannochloropsis salina* as a feedstock for anaerobic digestion. The objective was to quantity the volume of biogas that would be produced for treated and non-treated feedstock for anaerobic digestion. Concentrations of total nitrogen and ammonia were analyzed in treated and non-treated effluent and feedstock. At the end of the 30 day residence time, non-pretreated biogas amounted to an average of 725.04 ml (.725 L) of biogas per day, and for pre-treated feedstock there was an average of 725.04 ml (.725 L) of biogas was produced per day. Gas production values were taken from when the system reached a steady state gas production. There were higher amounts of nutrients in the pre-treated feedstock and effluent indicating some form of cell disruption. The results for these tests would suggest that the treatment did not successfully increase biogas production because of very similar steady state volume averages. This would require further analysis of biogas composition for continuing studies.

A suggestion would be to increase autoclave pre-treatment conditions to higher temperatures that would be high enough to biodegrade cell wall composition; thereby increasing biogas production.

Advisor: Dr. Edward Bouwer

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I would first like to give thanks to God because without Him none of these opportunities would have been possible. I would like to thank my advisor Dr. Edward Bouwer for his time, advice and fantastic teaching skills. I would especially like to thank Dr. Pavlo Bohutskyi for allowing me to be a part of his algae research group. This was an amazing opportunity to continue research in a field I am very passionate about which is renewable energy. I am very grateful for his time, advice and mentoring during my short time here. I would like to thank Steven Chow, Dean Yacar and Debora Cynamon Kligerman for all of their help and support and good times in lab.

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CHAPTER 1: INTRODUCTION

Global climate change is certainly one of the most controversial issues of our time which stems from the uncertain nature of the topic; however, what remains certain is the science behind of how the Earth functions. Throughout history, Earth's climate has varied due to complex interactions between oceans, winds, and land creating a cycle of warming and cooling for the last millions of years. Many think that climate change is a new concept not realizing that it is an intrinsic part of our planet's being. Although climate has said to been stable over the last 10,000 years, human impact has greatly influenced the Earth's climate. Sources of human impact include carbon emissions into the air from burning fossil fuels, deforestation, use of pesticides, and the list goes on. Climate change has been a direct response to the anthropogenic effects on greenhouse gases that are rapidly and constantly emitted into our atmosphere.

A major source of these greenhouse gases are a result of something that the human population could not live without; energy.

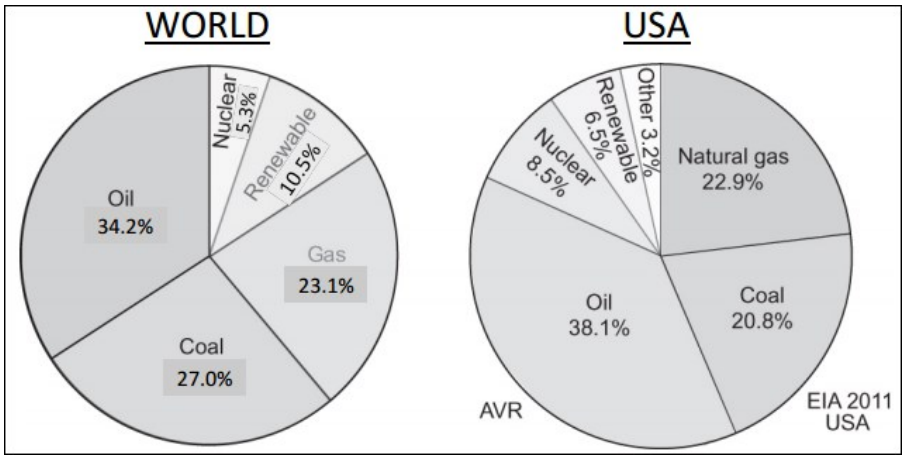

Figure 1.1:Energy sources (left) World, (right) United States
(Da Rosa, 2009)

Data from the Energy Information Administration plots energy resources for 2011. The majority of the energy needs come from finite sources that have left many negative impacts on the environment. Conventional energy includes finite resources such as coal, petroleum and natural gas. They are attractive because they are seen as efficient, dependable and have been engrained to be considered the most economical. Renewable energy is gaining much attention and research because of the detrimental environmental effects of theses conventional resources have been investigated and proven. Renewable sources could also positively impact political, economic and environmental sectors.

Renewable energy has the potential to limit the dependency on foreign countries for energy resources. From an economic standpoint, it could eliminate the cost of importing fossil fuels, tariffs, and cost of extraction thereby spurring infrastructure and development. Renewable energy developments would help to decrease greenhouse gas emissions and environmental pollution due to the effect of practices such as mining, fracking, and oil extraction.

One of the most promising renewable energy resources being investigated, researched and even used on a smaller scale has been biofuel technology. In a time in which finite sources such as fossil fuels are depleting, the reliance on other sources such as biofuels for energy use is ever increasing. Algae offer a promising feedstock for biofuel production because of its high growth rates, ability to thrive in extreme environments and high lipid content that can be converted to transesterfied biodiesel, fermented bioethanol, hydrocarbon biofuels, jet fuel, and biogas.

Biogas as the end product of anaerobic digestion of microalgae includes methane and carbon dioxide which can be used for electricity generation, heat, renewable natural gas,

and transportation fuels. Biogas can be generated from whole cell algae or algal residues. Biogas production can be seen as the most cost-efficient and environmental bioenergy technology as it can use organic waste and plant biomass as feedstock. Environmental benefits include mitigation of carbon dioxide through photosynthesis, bioremediation of wastewaters, and nutrient removal. One major limiting factor for this technology is microalgae cell wall biodegradability. In order to enhance the performance of anaerobic digestion, pre-treatment of microalgae is essential because of low cell wall biodegradability of non-treated microalgae.

This research was designed to be a preliminary investigation for the thermal pre-treatment of the very sought after microalgae species *Nannochloropsis salina* in order to test for biogas volumes produced.

CHAPTER 2: BACKGROUD AND LITERATURE REVIEW

2.1 Microalgae

Microalgae are a microscopic algae and cyanobacteria that use sunlight and atmospheric carbon dioxide for their source for growth by photosynthesis. The have a doubling time of 3.5 to 24 hours in an exponential growth phase (Schwede, Rehman, Gerber, Theiss & Span, 2013).

2.1.1 Microalgae Composition

Microalgae are generally comprised of proteins (6-52%), lipids (7-23%) and carbohydrates (5-23%) (Keymer, Ruffell, Pratt, & Lant, 2013). These percentages vary throughout the species of algae, and also is affected by environmental conditions. For several species, the high proportion of proteins is characterized by a low carbon to nitrogen ratio especially if compared with terrestrial plants. This ratio has an average of 10.2 for freshwater microalgae while it is 36 for terrestrial plants (Ketmer et al., 2013).

2.1.2 Nannochloropsis

Nannochloropsis is a genus of algae that is comprised of six known species. They have a size that ranges from 2-3 micrometers. They are typically found in marine waters, but can also be found in fresh and brackish waters. What makes them unique in relation to other microalgae is that they have chlorophyll A and completely lack chlorophyll B and chlorophyll C (AquaCare GmbH & Co. KG, 2010). What makes them of interest specifically for biofuels is their ability to accumulate high levels of poly unsaturated fatty acids.

2.2 Algae for Biofuel Production

2.2.1 Microalgae Biofuels

Microalgae have been heavily considered as a renewable source of energy both in the liquid and gaseous phase, and if made sustainable can compete with its fossil fuel contenders. As a whole, it is viewed as one of the most promising biofuel feedstock. Algal biofuel technologies are promising for lower greenhouse gas emissions as they offer a practical means for carbon capture (Zhao, Jingweir, Zhao, Laurens, Jarvis, Chen & Frear, 2014). Some species of microalgae can accumulate more than 50% of their dry weight in the form of lipids which can be extracted and converted into biodiesels (Cai, Park, Racharaks & Li, 2013). With high growth rate and triacylglycerols (TAG) lipid content, some algae offer an even greater potential for renewable energy feedstock. Lipids from algae can offer a variety of biofuel options including transesterfied biodiesel, fermented bioethanol, photo-biological hydrogen, hydrocarbon biofuels, diesel, and jet fuel (Zhao et al., 2014). Biogas can be generated from whole cell algae or algal residues. Biogas production is often seen as the most cost-efficient and environmental bioenergy technology.

2.2.2 United States' Biofuels Statistics

The United States' biofuels policy is reflected in the Renewable Fuels Standards (RFS) of the Energy Independence and Security Act of 2007. RFE mandates a biofuels production target of 26 billion gallons years (BGY) by 2022 (Pate, Klise & Wu, 2011). Consumption of liquid fuels in the US in 2008 from all sources was approximately 19.5 million barrels per day (MBD), over half derived from imported petroleum. This number is projected to

increase 2.5 MBD by 2035, with the fraction used from transportation increasing from 71% to 74% (Pate et al., 2011).

2.2.3 Biofuel Comparisons

Compared to terrestrial oil seed crops, microalgae produce 20 times more oil per area (Cai et al., 2013). The environmental benefits include mitigation CO_2 through photosynthesis, bioremediation of wastewater, removing large amounts of nutrients and heavy metals. Economically feasible and sustainable energy production from microalgae requires optimization for algal growth, maximization of lipid content and enhancement of biomass conversion into energy.

2.3 Anaerobic Digestion for Renewable Energy

2.3.1 Anaerobic Digestion

Anaerobic digestion (AD) is a biochemical process which typically runs with lower temperatures and has lower reaction rates than thermochemical technologies. It is more conducive to higher moisture feedstocks and biodegradable organic matter. Anaerobic digestion can come in a single stage and two-stage process. It has the added advantage of extracting intrinsic heat value, in the form of biogas for energy production from the feedstock (Monnet, 2003).

2.3.2 Anaerobic Digestion Process

Anaerobic digestion has a defined process flow that consists of four distinct phases. Anaerobic digestion is used to decompose organic matter under oxygen free conditions to produce biogas. Hydrolysis includes the breakdown of insoluble organic polymers such as carbohydrates to be available for bacteria. During acidogenesis bacteria convert sugars and amino acids into CO_2, H_2, and NH_3, and organic acids. During acetogenesis, bacteria can convert organic acids into acetic acid, ammonia, and hydrogen and carbon dioxide. During methanogenesis, methanogens convert these products into methane and carbon dioxide. These end products can be used for combustion to generate electricity and heat. They can also be used for renewable natural gas and transportation fuels.

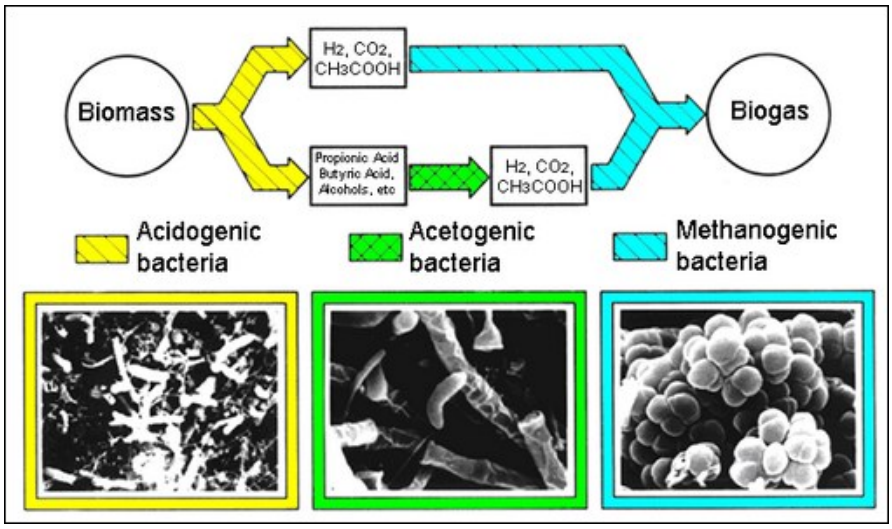

Figure 2.1: Schematic representation of the course of anaerobic methane generation from complex organic substances showing scanning electron micrographs of individual microorganisms involved (Serna, 2009)

2.3.2 Anaerobic Digestion of Microalgae-*Nannochloropsis salina*

Nannochloropsis salina is looked at as an exemplary species for anaerobic digestion because of its high biomass productivity and its high lipid content (Cai et al., 2013). It also has very high growth rates in northern Europe. Many microalgae are composed of algeanans which are insoluble, non-hydrolysable aliphatic biomacromolecules (Schwede et al., 2011). N. salina belongs to the class Eustigmatophytes which biosytnthesizes high amounts of algaenan. Of its composition, 1-2% of dry matter is detected as algeanans (Schwede et al., 2011). N. salina can also be cultured using AD effluent as a nutrient source for simultaneous lipid production and nutrient removal (Cai et al., 2013).

Effluent from AD contains high amounts of nutrients, such as ammonium and phosphate. For other species, levels of inhibitors such as ammonia in AD effluent is usually too high to be tolerated by the microalgae in anaerobic digestion. Although considered a model species, cell wall degradation of N. salina is a limiting factor which is also common for other species.

2.4 Anaerobic Digestion End Products

2.4.1 Biogas

The two main components of anaerobic digestion biogas are carbon dioxide and methane. Methane consisting of 55-70% and carbon dioxide consisting of 30-40% (Bohutskyi & Bouwer, 2013). Other minor components of this biogas mixture include small quantities of nitrogen, hydrogen, hydrogen sulfide, oxygen, sulfide compounds, siloxanes and volatile

organic compounds (Bohutskyi & Bouwer, 2013). Higher lipid content of the cell corresponds to higher the potential for methane yield. Some key parameters that could affect this biogas yield are hydraulic retention time and loading rate . They should be adjusted to allow the active populations to remain in the reactor to produce the maximum yield. An important factor that is observed for biogas quality is pH because it controls this carbonate system and the release of CO_2. High pH due to high alkalinity from ammonia release suggests that gas content will shift more to methane (Zhao et al., 2014). AD enhancement can come from observing composition of organic substrates for a better methane conversion yield.

2.4.2 Biogas Purification

Compared to other organic substrates, studies dealing with AD of algae are scarce. Quality of biogas is a key issue for longevity and efficiency of the thermic process converting methane to energy (Sialve, Bernet & Bernard, 2009). As previously mentioned, biogas contains small amounts of hydrogen sulfides, hydrogen, and other volatile compounds. Algae specie and composition also play an important role in the fate of the biogas composition. Biogas purification can be very efficient increasing certain components such as methane, over the others. Some studies have shown that algae can actually reduce the cost associated with biogas filtration.

2.5 Advantages of Microalgae Anaerobic Digestion

AD of biomass will be an essential component of the algal biofuel production system (Keymer, Ruffell, Pratt, & Lant, 2013). Microalgae are seen as one of the most promising

and sustainable biofuel feedstock. Their high growth rates and ability to thrive in harsh environments such seawater, wastewaters, arid and barren land makes them even more attractive. Microalgae coupled with anaerobic digestion are considered economical for methane production for low cost and low quality algal biomass that can be generated during wastewater treatment or harvested from water bodies (Keymer et al., 2013). Biogas production with anaerobic digestions avoids energy intensive steps including drying and extraction which in theory would make the process more economically feasible; however, there are some challenges to overcome.

2.6 Challenges with Microalgae Anaerobic Digestion

Composition of the cell wall is a major limiting factor that can result in low biodegradability for anaerobic digestion (Schwede et al., 2013). Resistance of the algal cell wall is generally a factor for cell digestibility therefore, hydrolysis of algal cells is the rate-limiting factor in AD for algal biomass. Many green microalgae have trilaminar outer cell walls (TLS) with high resistance to chemical and enzymatic degradation (Schwede et al., 2011).

As previously mentioned, AD can solve waste issues as well as economical and energetic balances; however, there are three main problems that may hinder its progress for scaling up in the energy industry. Three main bottlenecks include biodegradability of microalgae, high cellular protein content as a result of ammonia release which could lead to toxicity, and presence of sodium for marine species can also affect the digester performance (Sialve et al., 2009). A point to consider is that microalgae would involve huge quantities of

nitrogen and phosphate for which environmental and economic impact may not be sustainable; however, there are processes to recycle nitrogen and phosphorus contained in algal waste thereby reducing the use of fertilizers. AD can be the answer to this problem by mineralizing algal waste containing organic nitrogen and phosphorous.

Providing adequate CO_2 resources for enhanced algae production appears to be the biggest challenge in scaling up biofuel production (Pate, Klise & Wu, 2011). Few investigations have tried to explore the extent to which algae-based biofuels might reasonably be expected to contribute to US energy supplies.

2.7 Pre-treatment: Maximizing Anaerobic Digestion Yield

2.7.1 Pre-treatment of Microalgae for Anaerobic Digestion

Pre-treatment aims to rupture the cell wall, reduce the size of the particulate matter and crystallinity of the structural material, and to hydrolyze bimolecular polymers in order to maximize the results of anaerobic digestion (Bohutskyi, Bentenbaugh & Bouwer, 2013). Separation techniques include concentration, dehydration, and chemical treatments including acid, bases, and ozonation. Other methods include thermal treatment and ultrasonic lysis. Studies have shown that chemical pre-treatment alone is not effective for N. salina and Chorella because the thick polysaccharide based cell walls that these species contain (Bohutskyi et al., 2013). Thermochemical pre-treatment has been tested as a better option for these species as well.

An powerful alternative to pre-treatment is improving the digestibility of waste activated sludge, but not yet tested on algae is high pressure thermal hydrolysis (HPTH), which is certainly an option for future studies (Keymer et al., 2013). Pre-treatment is essential

because of low biodegradability of original composition of cells. Pre-treatment makes the organic matter more accessible to the anaerobic microflora and therefore more easily degraded.

2.7.2 Anaerobic Digestion and Pre-treatment of a Filamentous algae

Filamentous algae species are easier and less expensive to harvest as compared to unicellular forms. The have the potential to improve energy generation economics. Rhizoclonium has been studied as a leader in this type of biomass by applying different pretreatments to this species. Using filamentous algae could eliminate the energy deficiency as a result of culturing, drying and disrupting the microalgae (Ehimen, Holm-Nielsen, Poulsen, & Boelmand, 2013).

2.7.3 Anaerobic Digestion and Pre-treatment of *Nannochloropsis salina*

One area of investigation for thermal pre-treatment of N. salina is by heating in a drying cabinet in order to biodegrade cell walls. The results of this study suggest increase in absorbance from sampling, thereby indicating release of organic compounds such as proteins, carbohydrates and DNA signifying cell breakdown. This study also showed higher methane yields, three times higher than untreated samples. Batch assay confirmed the structure resistance of the algal cell wall is distinctively limiting the biodegradability of N. salina to biogas (Schwede et al., 2013). It suggests damaged cell walls provide a target for hydrolytic enzymes in the degradation process.

2.8 Research Objectives

The purpose of this experiment was to be a preliminary study to test the effects of thermal pre-treatment by autoclaving for *Nannochloropsis salina* as a feedstock for anaerobic digestion. The objective was to quantity the volume of biogas that will be produced for treated and non-treated feedstock for anaerobic digestion. The hypothesis was that thermal pre-treatment would increase biogas production because elevated temperatures should in some way disrupt the composition of microalga cell walls; more specifically within N. salina; thereby releasing nutrients that would aid in enhancing anaerobic digestion for biogas production. These experiments would also examine and compare the nutrients total nitrogen and ammonia, which are valuable components for methane production through anaerobic digestion.

CHAPTER 3: MATERIALS AND METHODS

3.1 Microalgae biomass

The industrially produced biomass *Nannochloropsis salina* was used as substrate for semi-continuous anaerobic digestion in the current study. This *Nannochloropsis salina* used for this experiment was provided by heliea® Incorporated.

3.2 Anaerobic Digestion Feedstock

3.2.1 Anaerobic Digestion Feedstock Preparation

The AD feedstock solutions were prepared in 2 L batches by diluting the frozen whole biomass residue with Milli-Q® water to obtain desired volatile solid content of 20 g VS/L. The feedstock solutions were stored at 4°C and pre-heated to 30-40 4°C by microwaving for approximately 1 minute prior to feeding. The manual withdrawal of the digested algae and addition of the algal feedstock was done once a day using 60 ml syringe. All samples were stored in a -20°C freezer until use.

3.2.2 *Nannochloropsis Salina* Feedstock Preparation

Nannochloropsis salina feedstock was prepared by adding 1 L of Milli-Q® water in a high-speed blender. N. salina residue was homogenized for 10 minutes using a blender equipped with 25 mm radius blade at 27k rpm. The mixture was measured out in a graduated cylinder and poured into 3L flask. Milli-Q® water is added such that the total mixture has a volume of 3L. The flask was left overnight -20°C freezer until to allow the powder to saturate itself in the water.

3.3 Experimental Set-Up

3.3.1 Anaerobic Digestion System and Operation

The anaerobic digestion experiment was carried out in a semi-continuous stirred tank reactor using a 3 L spinner flasks (Bellco Glass, Inc) containing 2 L of the anaerobic culture. The bioreactors were equipped with four sampling ports for solution withdrawal, feeding, and biogas collection. The AD systems were operated in the controlled environmental chamber at 35 ± 1° C and continuously mixed with an externally mounted variable-speed motor that rotated a stainless steel shaft with 12x15 cm (HxD) impellers.

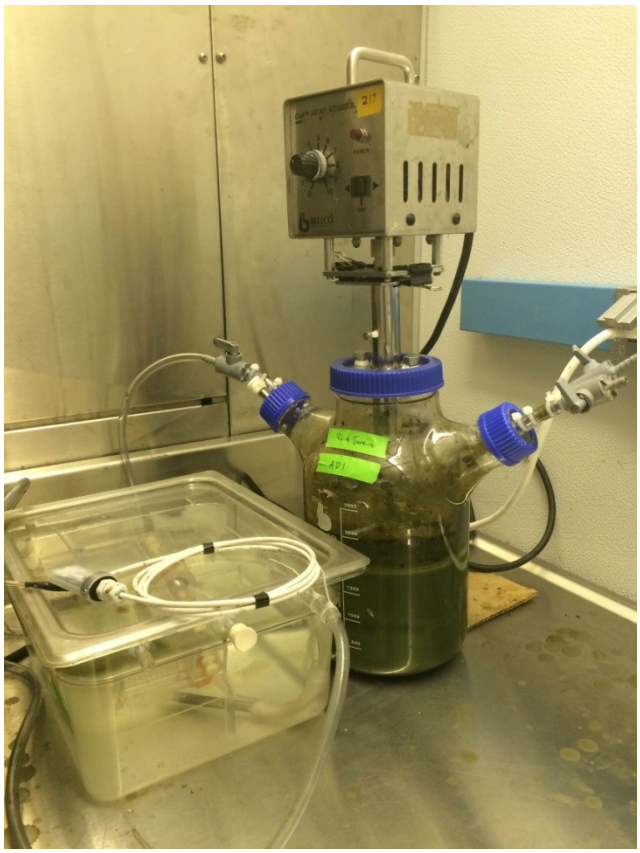

Figure 3.1: Anaerobic Digestion Stirred Tank Reactor and Gas Meter

3.4 Biogas Measurement

The volume of the produced biogas was measured using wet-tip gas meters (Wet Tip Gas Meter Co., Nashville, TN) equipped with a HOBO Pendant® G Data Logger (Onset Computer Corporation, Bourne, MA). Each flip of the monitor is calibrated to amount to 90.63 mL of biogas.

3.5 Thermal Pre-treatment

N. salina feedstock solutions were diluted to 20 g VS/L then were autoclaved at 121°C and 10 bar for 30 minutes. The treated samples were cooled to ambient temperatures then stored in a 20°C freezer until use.

3.6 Nutrient Analysis

After thermal pre-treatment, 30 mL samples were used for nutrient analysis. Anaerobic digestion treated and non-treated effluents were collect at the end of each residence time for nutrient analysis.

The analysis of the nutrient content in the liquid phase of the digestate samples were thawed at room temperature then centrifuged in 50 mL tubes at 4,200 rpm (4,000×g) at 4 °C for 10 minutes. The supernatants were collected and stored in a −20-°C freezer until nutrient analysis could be performed. All data points were generated in duplicate and reported as the average with standard deviations provided.

Treated and non-treated *Nannochloropsis salina* AD feedstock and treated and non-treated AD effluent were analyzed for total nitrogen (TN) and ammonia nitrogen (NH_3-N) using HACH kits. TNT 827 HR 5-40 mg/L TN was used to measure Total Nitrogen (TN)

concentration and TNT 832 HR 2-40 mg/L NH_3-N was to measure Ammonia Nitrogen (NH_3-N) concentration.

3.7 Analytical Techniques

Algal biomass was analyzed to determine total solids (TS) and volatile solids (VS) according to the Standard Methods for the Examination of Water and Wastewater (Eaton, Clesceri, Greenberg & Franson, 2005).

CHAPTER 4: RESULTS AND DICUSSION

4.1 Thermal Pre-treatment

4.1.1 Non-treated AD Feedstock

The anaerobic digester was given 100 mL of N. salina feedstock and 100mL of effluent was taken out each day to maintain an organic loading rate of 1.0 gVS/L-d over a hydraulic residence time of 20-30 days.

Figure 4.1 shows the gas production until a steady state was reached, which are the values in which gas production data was quantified. The steady state values are defined by a period of time when gas production values were constant. This signifies minimal fluctuations in the AD system thereby reporting the most significant gas production values. At the end of the 30 day residence time, non-pretreated biogas amounted to an average of 725.04 ml (.725 L) of biogas per day.

4.1.2 Pre-Treated AD Feedstock

At the end of the 30 day residence time for pre-treated feedstock, an average of 725.04 ml (.725 L) of biogas was produced per day. Figure 4.3 shows the comparison of biogas production data of both treated and non-treated feedstock over the 30 day residence time. Looking at daily biogas production, it shows that the pre-treated AD cycle have higher quantities of biogas production by volume as compared to the un-treated cycle data. Factors that could cause instability in the AD process includes and is not limited to fluctuation in operational parameters (T, pH, OLR, gas leakage). Presence of inhibitory compounds such as ammonia, sulfide, organic compound, metals, solvent residues, also play a role in AD performance as well.

These results would suggest that the treatment did not successfully increase biogas production because of very similar steady state volume averages. This would require further analysis of biogas composition for continuing studies.

A suggestion would be to increase autoclave pre-treatment conditions to higher temperatures that would be high enough to biodegrade cell wall composition. Previous studies, such as autoclave pre-treatment suggest that this type of treatment has moderate effect on N. salina but has a greater effect compared to other treatments such as chemical pre-treatment.

Hydrolysis of structural polysaccharides such as cellulose requires higher temperatures but partial solubilization of hemicellulose is possible at the study conditions. The high pressure applied during the thermal pretreatment likely did not facilitate cell disruption as well. Previous studies using autoclave thermal pre-treatment has test for biogas and methane yield using biomethane potential BMP test. Biogas and methane potential curves have been made from these types of test. Theoretical yields for methane have be recorded to be 0.63 L CH_4 $(g\ VS)^{-1}$ (Bohutskyi et al., 2014).

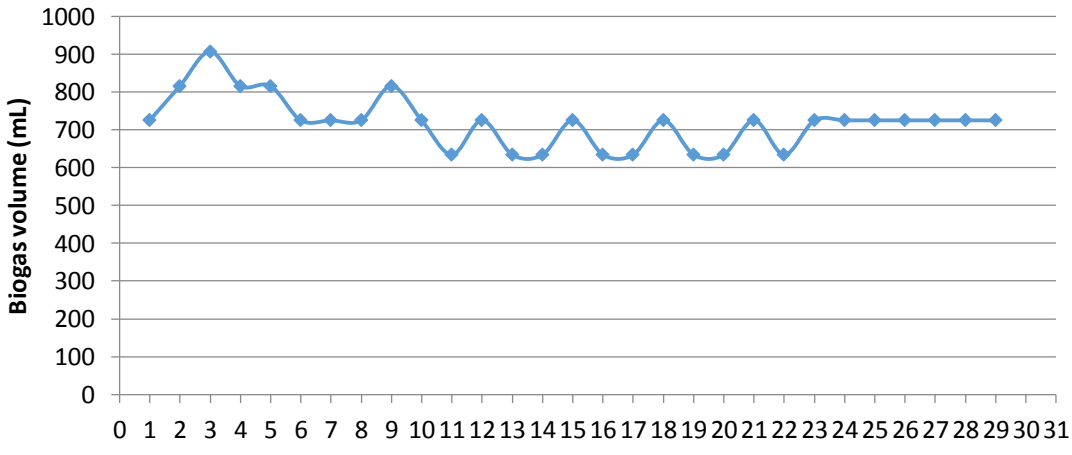

Figure 4.1: Biogas production of non-treated N. salina over 30 day residence time

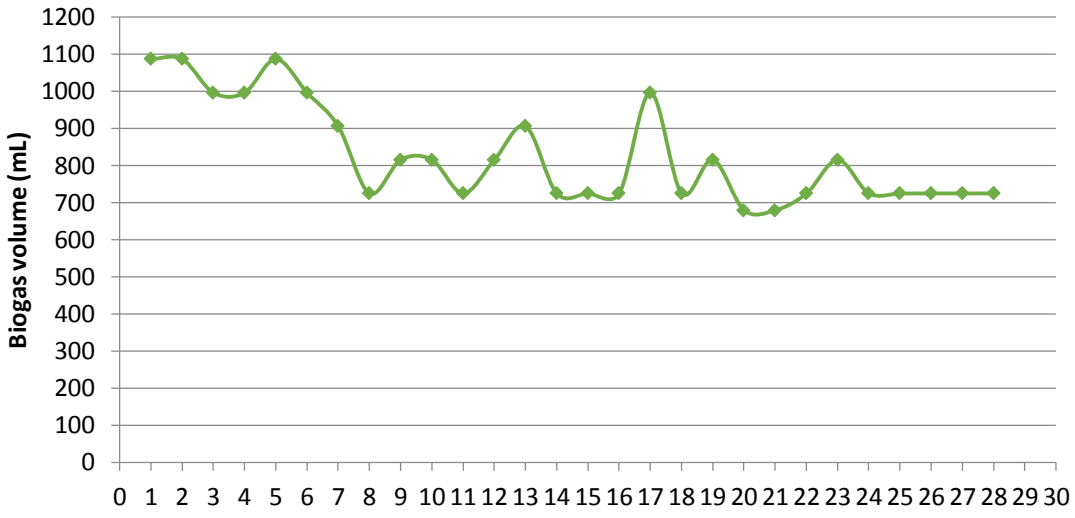

Figure 4.2 : Biogas production of thermal pretreated N. salina over 30 day residence time

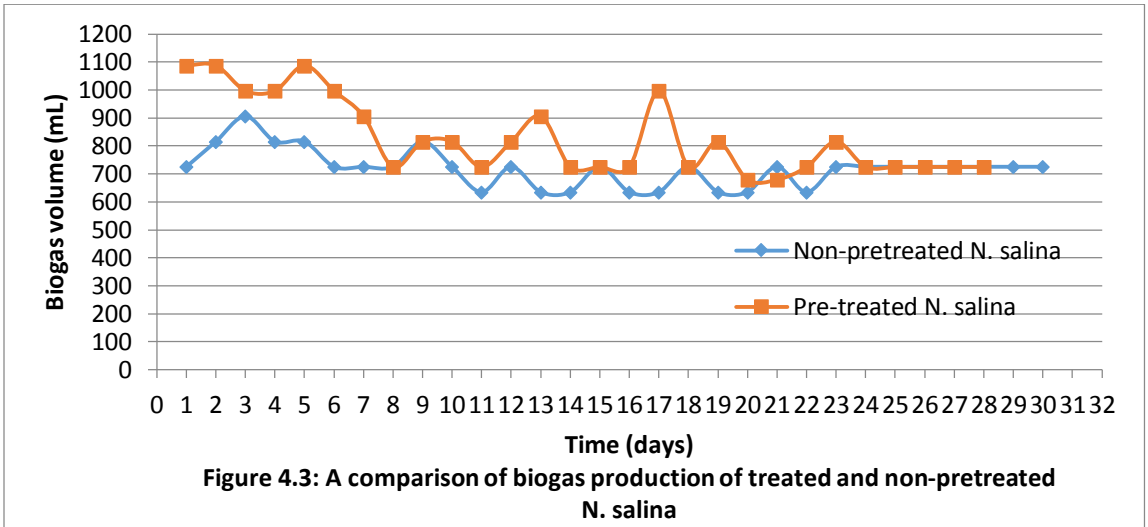

Figure 4.3: A comparison of biogas production of treated and non-pretreated N. salina

4.2 Nutrient Analysis

Treated and non-treated *Nannochloropsis salina* AD feedstock and treated and non-treated AD effluent were analyzed for total nitrogen (TN) and ammonia nitrogen (NH_3-N) using HACH kits.

4.2.1 Ammonia and Total Nitrogen

Table 4.1 Ammonia (NH_3-N) Concentrations

Sample	NH3-N (mg/L)	Average NH3-N (mg/L)	Standard deviation NH3-N (mg/L)
N. salina non-treated-1	39.01	37.67	1.89
N. salina non-treated-2	36.33		
N. salina 121C treated 1	49.84	50.53	0.98
N. salina 121C treated 2	51.22		
N. salina non-treated AD effluent-1	589.00	586.10	4.10
N. salina non-treated AD effluent-2	583.20		
N. salina 121 C treated effluent -1	586.54	590.22	5.20
N. salina 121 C treated effluent -2	593.90		

Table 4.2 Total Nitrogen (TN) Concentrations

Sample	Total TN (mg/L)	Average total TN (mg/L)	Standard deviation TN (mg/L)
N. salina non-treated-1	338.49	325.51	18.37
N. salina non-treated-2	312.52		
N. salina 121C treated 1	392.39	394.19	2.55
N. salina 121C treated 2	395.99		
N. salina non-treated AD effluent-1	697.11	705.24	11.50
N. salina non-treated AD effluent-2	713.37		
N. salina 121 C treated effluent -1	723.75	710.72	18.42
N. salina 121 C treated effluent -2	697.70		

The data shows the total nitrogen in the treated samples is higher than in the non-treated samples. This is expected and shows that the thermal pre-treatment did impact cell biodegradability in some way. It is noted that for ammonia, concentrations below 200 mg L^{-1} are beneficial for anaerobic processes (Schwede et al., 2013). During protein degradation in anaerobic digestion, ammonia is released and accumulates in solution (Schwede et al., 2013).

For the non-treated sample 11.5% of the total nitrogen was ammonia and for the treated sample 12.8% of total nitrogen was ammonia. For the non-treated effluent 83.1% total nitrogen was ammonia. For the treated effluent 83.0% total nitrogen was ammonia. This

high percentage of ammonia in the effluent has implications for nutrient recycling which has been done in other studies.

Biochemical composition of algal biomass has a major role on the methane potential Algae with high carbohydrates and protein contents are theoretically poorer substrates for methane production compared to lipid rich algae. Lipid rich algae have more carbon and hydrogens which could contribute to higher methane production.

CHAPTER 5: CONCLUSIONS AND FUTURE RESEARCH

5.1 Conclusion

This was a preliminary set of experiments to test the effects of thermal pre-treatment (autoclaving at 121°C) on biogas production for anaerobic digestion. Previous studies using a thermal pre-treatment have used BMP test to quantify biogas and methane production. Other thermal methods that have been studied have used heating cabinets at high temperatures for extended intervals of time. Species such as *Nannochloropsis salina* are sought after because of its high growth rates and high lipid content. Major barriers for using these microalgae species are biodegrading the cell walls to release nutrients for anaerobic digestion. *Nannochloropsis salina* possesses a thick and multilayered cell wall composed of polysaccharides with the presence of non-hydrolysable biopolymer; algaenan (Bohutskyi et al., 2013).

Based off of data from this study, thermal pre-treatment had little effect on biogas production when comparing to the non-pre-treated feedstock. These test did prove that nutrients were released from the pre-treatment from the nutrient analysis. These particular set of experimental parameters for biogas production with N. salina feedstock have only been tested with BMP test and have not been tested with semi-continuous anaerobic digestion until now. These test constituted as the appropriate first step into looking at N. salina under autoclave thermal pre-treatment conditions. The data from this experiment would suggest that increasing autoclaving temperature may improve the biogas production results.

5.2 Future Research

Previous studies report that chemical treatment has little effect on N. salina; however, thermochemical shows much potential. The next steps with this particular study would be to explore increasing autoclave temperatures. There should also be a means to analyze the biogas in order to quantify gas components such as with gas chromatography. It is also reported that with thermochemical treatment, biogas and methane yields achieved at the end of the incubation period and were enhanced up to 30–40% for species relative to the untreated control. When barriers such as cell biodegradability are overcome, we can look into scaling up these renewable energy resources that have gargantuan potential to be able to supply our energy needs; eventually rising to become a major contributor for energy demands right along with the finite fossil fuel resources.

APPENDIX

Table 4.1 Ammonia (NH_3-N) Concentrations

Sample	NH3-N (mg/L)	Average NH3-N (mg/L)	Standard deviation NH3-N (mg/L)
N. salina non-treated-1	39.01	37.67	1.89
N. salina non-treated-2	36.33		
N. salina 121C treated 1	49.84	50.53	0.98
N. salina 121C treated 2	51.22		
N. salina non-treated AD effluent-1	589.00	586.10	4.10
N. salina non-treated AD effluent-2	583.20		
N. salina 121 C treated effluent -1	586.54	590.22	5.20
N. salina 121 C treated effluent -2	593.90		

Table 4.2 Total Nitrogen (TN) Concentrations

Sample	Total TN (mg/L)	Average total TN (mg/L)	Standard deviation TN (mg/L)
N. salina non-treated-1	338.49	325.51	18.37
N. salina non-treated-2	312.52		
N. salina 121C treated 1	392.39	394.19	2.55
N. salina 121C treated 2	395.99		
N. salina non-treated AD effluent-1	697.11	705.24	11.50
N. salina non-treated AD effluent-2	713.37		
N. salina 121 C treated effluent -1	723.75	710.72	18.42
N. salina 121 C treated effluent -2	697.70		

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CURRICULUM VITAE

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EDUCATION

College of William and Mary (Williamsburg, VA**)** May 2013
Bachelor of Science, Major: Chemistry Second Major: Environmental Science and Policy
The Johns Hopkins University-Whiting School of Engineering (Baltimore, MD) December 2014
Masters of Science, Major: Geography and Environmental Engineering

WORK and RESEARCH EXPERIENCE

Chesapeake Bay Foundation, *Intern*, Richmond, Virginia
September 2008-August 2009
Organized volunteer events and contacted organizations for sponsorships
Tasked with organizing and checking all equipment for Grasses for the Masses Program

Chesapeake Bay Algae Project, *Research Assistant*, Williamsburg, Virginia
August 2009-May 2010
Worked with a professor in hopes to investigate a promising new technology
to produce biofuel from the algae growing naturally in rivers and the Chesapeake Bay
Learned how to use more advanced laboratory equipment

William and Mary Department of English, *Research Assistant*, Williamsburg, Virginia
August 2010-May 2013
Collaborate with a team of research assistants in maintaining the linguistics lab such as
compiling spreadsheets, organizing inventory, and contacting colleges and universities

William and Mary Department of Chemistry, *Research Assistant*, Williamsburg, Virginia
May 2012-May 2013
Worked closely with a professor in continuing research on project funded by Virginia
Institute of Marine Science (VIMS) to investigate mercury levels in fish

University of Botswana, *Research Assistant/Full Scholarship Recipient*, Gaborone, Botswana
May 2012-June 2012
NSF-IRES Research in Sustainable Energy for sub-Saharan Africa
Worked closely with a professor at the University of Botswana on a project to investigate
the potential for indigenous sweet sorghum as a source for biofuel production

William and Mary Department of Community Studies, *Teaching Assistant*, Williamsburg, Virginia
August 2012-December 2012
Assisted in CMST 250: African-American English, in supervising students interested
in STEM subjects

William and Mary Department of Environmental Science, *Teaching Assistant*, Williamsburg, Virginia
January 2012- May 2013
Assisted students in ENSP course, Sustainable Agriculture

Maryland Department of the Environment, *Intern*, Baltimore, Maryland
January 2013- May 2014

Designed a research project comparing environmental impacts of adopting various newer technologies for waste in Maryland - anaerobic digestion, gasification, waste to energy, and other conversion technologies. Made recommendations that could be used as support for any future policy related to waste portfolios, zero waste strategies, and future regulations that may address these technologies

The Johns Hopkins University Department of Geography and Environmental Engineering, *Research Assistant*, Baltimore, MD
May 2014-Present
Work with the principal investigator for the *Sustainable Algal Biofuels Solution: Sourcing and Recycling Nutrients from Waste Treatment Processing* project part of the EPA's National Student Design Competition for Sustainability Focusing on People, Prosperity and the Planet

PUBLICATIONS

Pavlo Bohutskyi, Benjamin Ketter, Steven Chow, **Kameron Adams**, Michael J Betenbaugh, F. C. Thomas Allnutt, Edward J Bouwer, 2014. Anaerobic digestion of residual Auxenochlorella protothecoides biomass after lipid extraction for methane generation and nutrient recovery, Bioresource Technology *(in review)*

VOLUNTEER EXPERIENCE

Environmental Defense Fund, *Regional Ambassador*, Washington D.C and Richmond, VA
July 2011-Present
Worked with the EDF on their national climate policy in order to mobilize legislators, strategic partners and grassroots groups to build support for strong clean energy and climate legislation in the national arena
Tasked with writing letters to Congress, letters to the editor, social media outreach, and contacting local environmental and grassroots organizations on climate policy issues
Kenya Sustainability Project, *Non-Traveling Member,* Williamsburg, VA and Kenya
October 2011-May 2013
Collaborated with a team of 5 other students who will work with the Children of God Relief Initiative (Nyumbani), a three-branched HIV/AIDS relief organization based out of Nairobi, Kenya
Fundraised to support traveling members and to increase donations available for the village

LEADERSHIP EXPERIENCE

WM NAACP, *Co-President,* Williamsburg, VA
August 2011-May 2013
Direct activities and meetings. Conduct educational conversations on diversity for Freshman Orientation. Write articles and act as a representative for interviews. Directs annual Dr. Martin Luther King march
Kappa Pi Chapter, Alpha Phi Alpha Fraternity Inc., Miss Black & Gold 2010-2011
December 2010-February 2012
Dedicated to diversifying the environmental movement and spreading overall awareness
Applied for the Alpha Green Initiative grant to fund a community garden for the WM NPHC on campus
Girls' World Forum 2012, *Adult Chaperone*, Chicago, Illinois
July 2012
Selected to represent as chaperone for the Virginia Commonwealth Girl Scout Council at Girl Scout and Girl Guide World Forum 2012 in Chicago, Illinois
Assisting girls on a "Take Action" project that aligns with the UN's Millennium Development Goals

American Chemical Society Rocky Mountain Regional Meeting, *Presenter*, Denver, CO
October 2012

Awarded funding to present research that was conducted at the University of Botswana

Alpha Kappa Alpha Sorority, Incorporated, *Connections Chairmen*, Williamsburg, VA
November 2012-May 2013
Responsible for providing chapter with information on issues of national, state, and local importance

UNDERGRADAUTE ACTIVITIES

Barrett Hall Council Representative	August 2009-May 2010
Student Leadership Foundation	Spring 2010
Freshman Orientation Aide	August 2010/2011/2012
NAACP, Public Relations Committee Chair	August 2010-May 2011
Rites of Passage, Mentor	August 2009- September 2010
Pearl of Great Price, Mentor and workshop facilitator	January 2011-Present

SKILLS

Languages: Proficient in Spanish and Italian
Computer: Proficient in Microsoft Office Suite, PC operating systems
Techniques: Mercury Analyzer Program, GC-MS, ICPMS

HONORS & AWARDS

Ewell Award, Dean Hardy Trailblazer Award, NSF-IRES Program on Research in Sustainable Energy for Sub-Saharan Africa, William and Mary Scholar, Miss Black and Gold Scholarship 2010-2011, Girl Scout Gold Award, National Delegate Representative at 51st National Girl Scouts of America Convention, Chesapeake Water Environmental Association Student Paper Competition 3rd Place Winner